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ARTICLE TYPE

Evidence of chitosan-mediated reduction of Au(III) to Au(0) nanoparticles under electron beam by using OH· and e⁻aq scavengers

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Selective scavengers of e⁻aq and OH· radical were used to
10 **investigate the radiolytic synthesis of gold nanoparticles from Au(III) solutions in the presence of chitosan. This reaction does not exclusively follow the direct reduction by solvated electrons. Irradiation generates short-lived and long-lived reductive species derived from chitosan that efficiently**
15 **convert Au(III) into Au(0) which aggregate to form clusters.**

Because of their unique optical, electrical, and chemical properties, gold nanoparticles (AuNps) continue to receive considerable attention due to the complex processes taking place during their synthesis as well as because of their broad range of
20 potential applications[1-3]. The two distinct features of the synthesis generally relate (i) to the reduction method by various direct or indirect redox agents and (ii) to the colloidal stabilization by polymers or surfactants[4-6], the so-called capping agents.[7-9] Alternatively, the use of ionizing radiation has been established
25 as a promising method for the synthesis of metal NPs, with reactive species exhibiting high reduction potential generated *in situ*.[10-13] The radiolysis of water generates a variety of species ((e^-_{aq}), H_3O^+, H·, OH·, H_2, H_2O_2), which include hydrated electrons (e^-_{aq}) and hydrogen atoms which can easily reduce metal ions,
30 including Au(III), down to the zero-valent state.The reducing species can be uniformly distributed in the solution yielding metal nanoparticles evenly dispersed with possible control of their size by varying the irradiation dose and dose rate.

This study deals with the formation of AuNps in the presence of
35 chitosan. A recent report showed that gold speciation influences the size and morphology of Au colloids.[13] Clarification of the reduction mechanism of Au(III) to zero-valent gold is a fundamental issue of interest and would help to control the formation of nanoparticles in terms of shape and dimensions. The
40 interaction of Au(III) with reducing species generated from water radiolysis, leading first to the formation of Au(0) clusters, and then of AuNps, has been established by the pioneering research of Belloni *et al.,*[10,11] Henglein,[12] and Meisel *et al.*[14] (Scheme 1). However, the influence of chitosan, a less conventional capping

45 agent, on the reduction process has not been specifically addressed.[15,16] Questions arise as to what degree is the possible contribution of chitosan to the reduction process and how to assess its influence on this pathway with respect to direct reduction by the species generated by water radiolysis.

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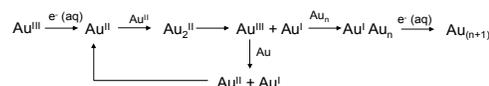
Scheme 1: Radiolytic route for the reduction of aqueous Au(III)

Of particular interest was whether a template effect could influence the formation of metal clusters during the initial stages of nanoparticles formation. This can be due to the polymeric
55 nature of chitosan and to the complexation of gold ions by glucosamine units through N,O coordination. This was shown in a recent study based on X-ray absorption spectroscopy.[17] Complex effects may affect the reduction of Au(III), the formation of nascent Au(0) clusters as well as their evolution
60 into nanoparticles. Taking into account these mechanistic considerations, we have assessed separately the contributions of primary species generated by radiolysis, on the one hand, and on the other, of those entities that would involve chitosan-based reductive groups, such as carbon-centered free radicals resulting
65 from H abstraction by OH· or other oxidative free radicals, or glucosamine (GAU) end-groups[18] (Scheme 2).
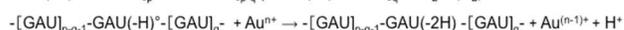
Scheme 2: Competing pathways of the radiation-induced reduction of aqueous Au(III) in the presence of chitosan (n=3,2,1) wherein GAU
70 stands for glucosamine unit.

The effects of EB irradiation on aqueous solutions containing chitosan (30 mM of glucosamine units (GAU)) and Au(III) ions (1 mM) introduced in the form of HAuCl₄ were examined. For

these experiments, a well-defined sample of purified chitosan exhibiting a deacetylation degree of 82% (determined by NMR) and a viscosimetric average molecular weight of 56000 was dissolved in aqueous acetic acid (166 mM), so as to obtain a final pH value of 4. AuNp formation was monitored by recording UV-visible spectra of irradiated $HAuCl_4$–chitosan solutions.

In the absence of specific scavengers and in an atmosphere of Ar, the irradiation of solutions of Au(III) and chitosan to a dose of 2.5 kGy induces the formation of Au-NPs revealed by the surface plasmon resonance band at 530 nm (A = 0.32) measured 19 h after the radiation treatment (Fig. 1-a).[3] Increasing the dose leads to the apparently surprising decrease of the absorbance together with a slight shift of the maximum towards shorter wavelengths. A similar observation has been reported for AuNps and AgNps generated radiolytically in THF solutions.[19] That phenomenon was explained by the precipitation of metallic Nps in the form of aggregates. In using high dose rates in these experiments involving chitosan (10 MeV EB, 500 Hz pulse frequency, average dose rate of 15 kGy s^{-1}) a very large number of germination sites were induced which lead to the formation of AuNps of much smaller size (typical average diameter < 5 nm). At this small size the particles do not reach the critical dimensions enabling the typical surface plasmon resonance to take place[3], as has been already reported by Akhavan *et al.*,[20] for AuNps stabilized by proteins. The immediate appearance of the red coloration by increasing the ionic strength of the EB-irradiated solutions upon NaCl addition supports the existence of a pool of Au clusters in the solution that aggregate to form Nps. Previous results obtained by γ-ray irradiation (at the low dose rate of 1.1 kGy.h^{-1}) of solutions having the same composition,[21] did not show significant changes in the plasmon resonance band once Au(III) salt reduction was completed.
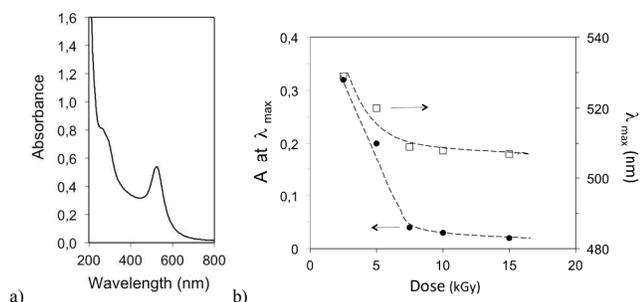
Fig. 1 a) Typical UV-visible spectrum of a Au(III)-chitosan solution irradiated under Ar ([Au] = 1 mM, [GAU] = 30 mM, 1 mm path), and b) influence of EB dose (2.5-15 kGy) on the main features of the plasmon resonance band recorded 19 h after irradiation (dashed lines are only guides for the eyes).

The mechanism of the reduction of Au(III)-chitosan solutions was further investigated by use of specific scavengers for the main species generated by water radiolysis, and/or by introducing the Au(III) ions after irradiation of the aqueous chitosan solutions. Experiments conducted in the presence of N_2O or 2-propanol allows for the discrimination between the direct reduction and the secondary reductive pathways which rely upon the indirect effects of primary species onto chitosan, by quenching e- aq and OH$^{•}$, respectively.[18,22] The efficacy of N_2O, as an electron scavenger was confirmed by the absence of significant change in the UV-vis spectrum of $HAuCl_4$ solutions containing no chitosan after EB irradiation (Fig. 2, curves a and b). The spectra show a single peak at 315 nm characteristic of square planar $AuCl_4^-$ species,[17] confirming that Au(III) reduction

does not take place at all. Moreover, the UV-vis spectra of $HAuCl_4$-chitosan in aerated or in N_2O saturated solutions show similar features in the 400-700 nm range after irradiation at 10 kGy (Fig. 2, curves c and d). Absorbance values observed between 250 and 350 nm is slightly higher. This can be due to oxidized moieties generated on chitosan chains or from the chains after scissioning. The significant absorbance value (A = 0.23) at 523 nm indicates that the reduction of Au(III) and the formation of gold nanoparticles take place even with efficient trapping of solvated e$^-$. In both cases, the concentration of the residual gold ions determined by ICP-OES on $HAuCl_4$-chitosan solution after aggregation of AuNps by addition of NaCl and ultracentrifugation was found negligible (less than 5% with respect to initial [Au] concentration). These data prove that reduction was driven almost to completion even under conditions suppressing the direct reduction mechanism. The mean diameter (*d*) of AuNps determined by dynamic light scattering measurements is equal to 9 nm under N_2O, and 7 nm under air (Fig. 2, insets e and f).
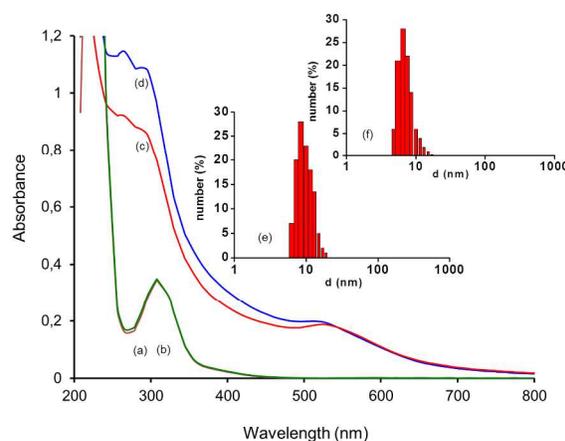
Fig. 2 UV-vis spectra (1 mm path) of 1 mM $HAuCl_4$ solutions: a) unirradiated, b) after 10 kGy irradiation under N_2O, c) after 10 kGy irradiation in the presence of chitosan [GAU] = 30 mM under N_2O, d) after 10 kGy irradiation in the presence of chitosan [GAU] = 30 mM without deaeration. Inset: Size distribution from DLS measurements of AuNps formed by irradiation at 10 kGy of $HAuCl_4$-chitosan solutions e) under air and f) under N_2O.

In the presence of 2-propanol, the primary free radicals resulting from radiolysis of Au(III)-chitosan solutions are expected to be quenched very efficiently, thus limiting, if not suppressing, the secondary reduction pathway involving chitosan-based intermediates.[10] Indeed, the spectra of Fig. 3. show that in the absence of OH$^{•}$ scavengers (Fig. 3-c), the application of a 5 kGy dose induces the formation of AuNps in high yield, as indicated by the absorbance at 523 nm and by the minor amount of residual Au(III) confirmed by ICP-OES measurements. In the presence of 2-propanol, at 5 kGy (Fig. 3-a), the absorbance of plasmon band is very weak, indicating that AuNps formation was prevented to a significant extent. Increasing the dose up to 15 kGy (Fig. 3-b) and to 50 kGy (not shown) enhances the absorbance which eventually reaches much lower levels than observed in Fig. 3-c. ICP-OES measurements confirmed that high doses are required (> 40 kGy) to achieve complete Au(III) reduction.

These observations lead to the conclusion that in the presence of chitosan, the radiolytic reduction of Au(III) to form AuNps mainly involves chitosan-based species formed *in situ*. Direct e-$_{aq}$ capture by free or chitosan-bound Au(III) takes place as a minor pathway, at least for the first step of the multi-step reduction.

Under the given conditions, Au(III) reduction is not controlled by the reduction potential of the primary species (e^-_{aq}) or secondary species ($(CH_3)_2C^{\bullet}OH$) generated *in situ*, but is submitted to some kinetic control caused by the complexation of Au(III) ions. For the same radiation dose, the size of AuNps is higher in the presence of 2-propanol, as shown in Fig. 3-e.
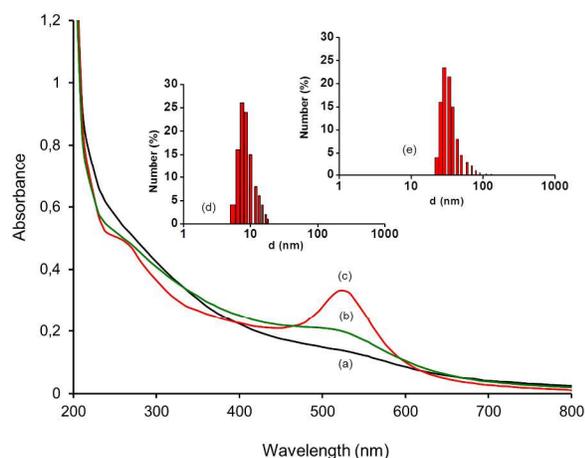
Fig. 3 UV-vis spectra (1 mm path) of irradiated HAuCl₄-chitosan solutions ([HAuCl₄] = 1 mM, [GAU] = 30 mM) a) in the presence of 130 mM 2-propanol at 5kGy, b) at 15 kGy, and c) in absence of 2-propanol at 5 kGy. Inset: Size distribution (DLS) of AuNps obtained d) after 15 kGy irradiation without 2-propanol, and e) with 130 mM 2-propanol.

At 15 kGy, the average diameter is 34.2 and 8.8 nm with and without 2-propanol, respectively. As reported earlier, chitosan binds Au(III) ions, possibly inducing a template effect which would influence the size of nascent AuNps formed during irradiation, as well as in the final state. Consequently, the size of these nascent AuNps may depend on the length of chitosan chains. The presence of 2-propanol limits H-abstraction from chitosan, and hence reduces scissioning. The resulting decrease influence on the average chain length of chitosan may act on the formation of nascent Au clusters of larger size, and finally result in larger AuNps.

The reductive properties of chitosan solutions irradiated under conditions similar to those of the previous experiments were shown by introducing Au(III) after the radiation treatment. When the Au salt is introduced in the unirradiated chitosan solution, the 400-700 nm wavelength range remains free of any new band, indicating that no significant amount of AuNps are formed within this time window. The development of gold nanoparticles requires more than 50 h of induction, the absorbance of the plasmon resonance band reaching a value of 0.05 after 5 days. The addition of HAuCl₄ to chitosan solution irradiated to 5 or 15 kGy, induces a light pinky red coloration, either immediately after irradiation or after a delay of 18 h, followed by the build-up of the absorption band around 520 nm attesting the formation of AuNps. The plots of Fig. 4 clearly show the efficacy of the irradiated chitosan solutions as a source of reductants, with a strong influence of radiation dose.

The formation of the long-lived species derived from chitosan is affected by the presence of 2-propanol during the irradiation step. This is exemplified by the spectra recorded 24 h after the addition of Au(III) ions to irradiated chitosan solutions (Fig. 5).
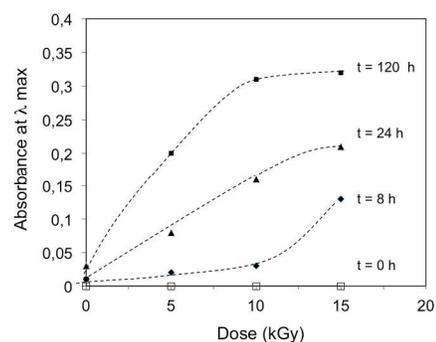
Fig. 4. Build-up of the surface plasmon resonance band at 520 nm recorded after various periods of time (t) following the deferred (18 h-delay) addition of Au(III) into unirradiated and irradiated chitosan solutions (0, 5, 10 and 15 kGy dose ; [HAuCl₄] = 1 mM ; [GAU] = 30 mM) ; dashed lines are only guides for the eyes).

In the absence of 2-propanol, the increase of absorbance at 265 and 285 nm with the radiation dose (Fig. 5-a, b, c, d) is consistent with the formation of carbonyl groups that can follow H-abstraction on glucosamine units.[22] This effect is almost absent in the presence of the OH• scavenger (Fig. 5-e).
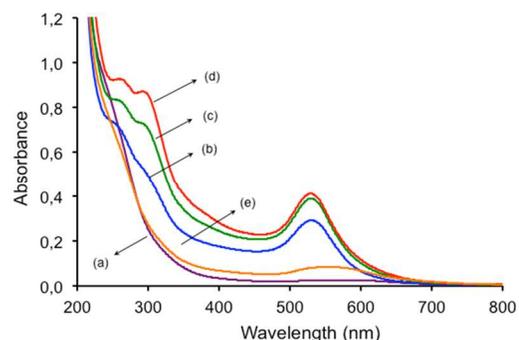
Fig. 5. UV-vis spectra (1 mm path) of chitosan solutions recorded 24 h after the addition of HAuCl₄ into the chitosan solutions: a) unirradiated, b) irradiated at 5 kGy, c) 10 kGy, d) 15 kGy and e) at 15 kGy in the presence 130 mM 2-propanol, [HAuCl₄] = 1 mM, [GAU] = 30 mM.

In summary, the main observations made in this study were:
- (i) unirradiated chitosan does not significantly reduce Au(III) ions in the used conditions and considered timeframe ;
- (ii) there is limited efficiency of N₂O with respect to AuNps formation during the irradiation of Au(III)-chitosan solutions indicating that the direct reduction by solvated e^- is not the main pathway ;
- (iii) the presence of 2-propanol decreases the reduction efficiency of chitosan-derived species towards Au(III) ions, in the two conditions used in this study, namely the simultaneous and the deferred reduction resulting from chitosan irradiation.

Chitosan shares with most other polysaccharides a high sensitivity towards the direct and indirect effects of high-energy radiation. Previous investigations based on molecular weight measurements and on spectroscopic data draw a reasonable picture of the events taking place during and after the irradiation.[18,24] The attack of OH• and H• radicals onto glucosamine units is known to result in H-abstraction mostly located at C1, C4, C5 carbon atoms. The formed free radicals can gradually reduce the Au ions to metallic gold via a one e^- process. The coordination of Au(III) ions to glucosamine reported earlier may have a favorable incidence on the first electron transfer via a template effect.

The much lower efficiency of gold salts reduction when the Au(III)-chitosan solutions are irradiated in the presence of 2-propanol confirms the minor contribution of the direct pathway involving solvated electrons. This says that the process is not under thermodynamic control directly dependent on the redox potential of the reductants. We believe that the strong binding of Au(III) ions to chitosan induces the non-homogeneous distribution of the Au(III) ions in the solution, and also creates a shield by steric hindrance around the cationic centers. This situation seems to favor the intramolecular electron transfer from the free radical sites located on chitosan to the coordinated metal ions. Similarly the shielding effect due to the formation of complexes likely explains the poor efficiency of the $(CH_3)_2C^•OH$ radicals towards coordinated Au(III).

In the absence of Au salts as a reducible species in the medium, the radiation-induced scission of the glucosidic bond in anoxic conditions is reported to follow the poorly selective formation of free radicals by H-abstraction at the different carbon atoms of glucosamine units, yielding a variety of terminal units, including the unmodified glucosamine end-units, as well as cyclic or open units with a carbonyl group[18,24] (Scheme 3). The formation of additional glucosamine end-groups generated by chitosan radiolysis could enhance the amount of reducing species with respect to the unirradiated chitosan where the concentration in reductive end groups is only 86 µM. On the basis of viscosity measurements, under similar conditions, the molar mass of irradiated aqueous chitosan at 5, 10 and 15 kGy are 6500, 5700 and 5200 g mol⁻¹, respectively, corresponding to an increase in end-groups by a factor ranging between 8 and 11. In order to evaluate this effect, the formation of AuNps in $HAuCl_4$-chitosan solutions by adding various amounts of glucosamine (from 0.15 to 17 mM). Au(III) reduction is very limited after 72 h, whatever the concentration of glucosamine, whereas, as already mentioned, reduction is noticeable 24 h after addition of the metal salt to the irradiated chitosan solutions. These observations rule out the influence of glucosamine reductive end-groups that would result from the chain scission of chitosan.
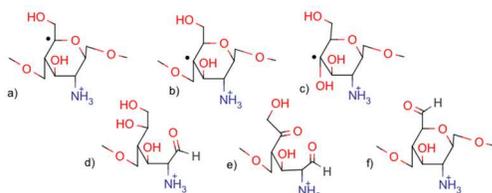
Scheme 3: Possible structures of short-lived (a-c) and long-lived (d-f) species derived from chitosan effective for reducing Au(III).

The influence of the presence of dioxygen during irradiation has also to be taken into consideration. Indeed, we have previously observed that, at similar radiation dose, the absorbance of plasmon resonance band is significantly higher in aerated solutions compared to the argon-saturated ones with the same composition. This raises questions both on contribution of peroxy derivatives to the formation of reductive moieties, and on the incidence of the lower degradation degree of chitosan, when irradiated in aerated solutions.[22]

In summary, it was shown that the role of chitosan is not restricted to the colloidal protection of AuNps. The conversion of Au(III) into AuNPS in the presence of chitosan does not exclusively follow the direct reduction pathways described in the literature. These results establish unambiguously the occurrence of a chitosan-mediated reduction process of Au(III) into Au(0) under e⁻ beam irradiation. This process involves primarily the formation of free radical by H-abstraction from chitosan chains resulting in scission. Both the short-lived and the long-lived species derived from chitosan are shown to effectively reduce Au(III) and to produce AuNps with a typical size ranging from 8 to 40 nm, depending on the conditions. Further work is currently in progress that is investigating the template effect that likely controls the mechanism of AuNPs formation. ESR experiments on model compounds should bring out useful information for characterizing the reductive short-lived species. The long-lived ones could be identified by advanced NMR analyses as well as by mass spectrometry.

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